# Enzymatic catalysis modelling

## Background

Endolysins are proteins used by phages in order to mediate the cell lysis needed for the virus to be released. Their action is similar to that of lysozymes, which are proteins that mediate the lysis of bacteria as a first line of defense in humans and other organisms. *Acinetobacter* phages have only recently been studied, therefore information is updated but still incomplete. To provide a mathematical model of the catalysis of the Abp phage endolysin (PlyAbp1) other models need to be studied, for this purpose lysozymes and other endolysins, such as λ phage, will be used as a basis.
The quantitative details of enzymatic lysis is relevant to a number of biotechnological applications including protein extraction, the engineering of transgenic livestock resistant to microbial infection, but in relation to the project it plays an important role in the design and assessment of therapeutic or antimicrobial treatments based on these phage-derived enzymes[1].

*Enzyme kinetics*
As an introduction prior to the catalysis model it is necessary to understand how enzyme catalysis and kinetics works. Enzymatic catalysis is defined as an enzyme (most of them proteins) that increases the rate of a certain reaction, this occurs when the energy of activation decreases by the action of the conformational change that develops when the enzyme and the substrate adhere into the entity called the enzyme–substrate intermediate.

Corresponding to the enzyme kinetics is important to remember that in biological systems several variables including biochemical, physical and physiological properties meddle with the production and degradation rates of reactions. In enzyme kinetics these reaction rates are measured and these changes in the rates are studied so the catalytic mechanisms of the enzyme can be understood. In this manner the role of the enzyme in the metabolism and how the enzyme activity is controlled can be fully explained.

## Modelling

The designed system formed by a fused holin - endolysin provides key advantages and factors to take into consideration:

- The amount of holin is directly proportional to the quantity of endolysin produced by the cell in a 1:1 relation. Quantification of the fusion protein will give provide immediate data of each protein.
- No antiholin means that there are no holin-antiholin dimers that may inhibit the action of holins and subsequently of endolysins. This also means that immediate accumulation of holin will take place once it is added to the medium.
- PlyAbp1 has no reported inhibitors or interactions due to the lack of deep study of the enzyme. For the moment any type of reversible or irreversible inhibition is neglected.
- Endolysins have only one substrate, then multiple substrate kinetics are not necessary.

[1] Mitchell,G. J., Nelson, D. C. & Weitz, J. S. (2010). Quantifying enzymatic lysis: estimating the combined effects of chemistry, physiology and physics. Retrieved from http://ecotheory.biology.gatech.edu/sites/default/files/gjb_dcn_jsw_physbio2010.pdf

Due to the previous considerations, the enzyme kinetics of the endolysin are likely to be governed by the Michaelis - Menten model. No specific kinetic parameters for the PlyAB1 endolysin have been reported to date. However, endolysins will not reach its substrate until the holin has formed the "hole" (or holes) in the membrane where endolysins can catalyse the lysis of the peptidoglycan layer. Holins do not catalyse any reaction, they only destabilize the membrane and eventually cause ion leakage and titrate out the membrane potential[2]. Then, holins are not to be analysed as enzymes, for which other models are needed to determine the amount of holins and time needed to provide access to the cell wall.

Some models have been proposed to explain how holins work:

- Energy poison model: it has already been discarded by experimental data [2].
- Critical concentration model, also known as "death raft" model[3]: holins accumulate until a determined two-dimensional concentration, number density or mole fraction is achieved. The hole begins to form once the critical amount is reached. If the hole is capable of disrupting the membrane potential then the whole holin action is triggered. This model basically considers that one hole will be formed, but it has been proposed that more than one may be produced and that the first to reach the critical concentration will be the one to trigger the lysis [2].
- Marginal Value Theorem: has also been employed successfully to calculate lysis times according to environmental, genetics, among other factors.

From the study of λ phage the following is known specifically about the S105 holin (canonical) which forms approximately the same number of holes and size as T4 phage holin T (the holin of choice for the project)[4]:

- Formed holes are stable: time does not define the size or distribution of the holes once the lysis has been triggered, in other words, they do not expand or contract. Hole formation is not the result of accumulated damage to the cell membrane that eventually spreads, it is a one-chance event.
- Hole size tends to small diameters with an average size of 340 nm, 1 μm of total hole perimeter and 2 holes per cell.
- At around 1000 holin molecules per cell the lysis response is triggered.
- *In vitro* - Forms ring-shaped structures that later constitute stacks of 640kDa mainly of ring-dimers. Each ring constituted by 18 - 20 protomers, each made of a λ phage holin tetramer. These rings have an outer diameter of 23nm, inner diameter of of 8.5nm, and height of 4nm.

The previous data should be taken with care due to the inconsistencies on lesion size given by *in vivo* experiments and *in vitro* assays. Literature is contradictory on whether lesions expand or once the critical concentration is reached a stable hole is formed, but the most accepted idea is that their size is constant. Nevertheless, this data is a good starting point for a lysis model. Mathematical models

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[2] Calendar, R. (n.d.). The Bacteriophages. Retrieved from https://books.google.com.mx/books?id=hYcRDAAAQBAJ&pg=PA116&lpg=PA116&dq=critical+holin+concentration&source=bl&ots=uqq2skk-uD&sig=cbcaDr5Wwx4Y9zv8BK-5IT-pLTs&hl=es-419&sa=X&ved=0ahUKEwjkh7SV6ZnNAhUk5oMKHXtuAa8Q6AEISjAE#v=onepage&q=critical%20holin%20concentration&f=false

[3] Krupovic, M. and Bamford, D. (2014). Holin of bacteriophage lambda: Structural insights into a membrane lesion. Retrieved https://www.researchgate.net/publication/5281580_Holin_of_bacteriophage_lambda_Structural_insights_into_a_membrane_lesion

[4] Savva, C., Jill, D., Moussa, S., To, K., Holzenburg, A. and Young, R. (2013). Stable micron-scale holes are a general feature of canonical holins. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/mmi.12439/full

regarding holins are rare but Singh and Dennehy[5] have proposed one for the production and accumulation of holins in the membrane. This model provides an insight on how bacteriophages cause lysis. The study includes mRNA transcription and translation rates, protein degradation considered as zero because holins and endolysins naturally accumulate in the cytoplasm until lysis starts, and parameters that take into account the randomness of holin aggregation. One important difference between this model and the basis of iGEM Tec CEM 2016 collegiate project is that host lysis, in this case *E. coli* is the production host, is not the most important parameter. What should be modelled is how fast *Acinetobacter baumannii* can be lysed using the holin-endolysin fusion previously purified from *E. coli*. This means that transcription and translation rates on *E. coli* are not relevant. Therefore, the most important variables are directly related to the enzyme kinetics described by Michaelis-Menten, which include the following:

- Enzyme concentration and amount: determined by the purification process, formulation, and amount of formula used.
- Substrate concentration: peptidoglycan can be considered as abundant, therefore it will not be a limiting step. It will simplify the Michaelis-Menten model.
- Affinity of the enzyme for its substrate ($K_M$): PlyAB1 is described as a highly specific endolysin for *A. baumannii*, then it must have a high affinity for its substrate (low $K_M$) which guarantees catalysis and not a stable ES complex with no reaction.
- Accessibility to the substrate: determined by the holin "death raft". Assumptions have to be made in order to generate one of two possible models. First, if the hole has a constant diameter, then only a certain quantity of enzyme can reach the cell wall and endolysin concentration will increase in a defined rate. Second, if the hole can grow in size it will allow endolysin to accumulate at an increasing rate. The latter will produce a faster lysis.

$$E + S \overset{k_1}{\leftrightarrow} ES \overset{k_2}{\rightarrow} P + E$$

**Figure 2.** The Michaelis - Menten model follow the kinetic enzymatic mechanism where E represents enzyme, S denotes substrate, P denotes product and ES denotes enzyme substrate complex. The ki 's are the rate constants.[6]

Three elements were taken into account to develop a first proposal for the enzymatic modelling of the project: Michaelis - Menten model, Heaviside function and Fick's Laws of Diffusion to develop two approaches..

*Michaelis - Menten*

The Michaelis - Menten model has the following equation:

$$V(t) = \frac{V_{max}[S]}{K_M + [S]} \quad (1)$$

where V(t) is the catalysis rate (or velocity), $V_{max}$ is the maximum catalysis rate, [S] is the spubstrate concentration, and $K_M$ is the affinity parameter also known as the concentration at which half of the $V_{max}$ is achieved (the Michaelis constant).

[5] Singh, A. and Dennehy, J. (2014). Stochastic holin expression can account for lysis time variation in the bacteriophage lambda. Retrieved from https://udel.edu/~absingh/Site/Publications_files/2014%20Singh%20and%20Dennehy.pdf

[6] Ozogur, S. (2005). MATHEMATICAL MODELLING OF ENZYMATIC REACTIONS, SIMULATION AND PARAMETER ESTIMATION. Retrieved from https://etd.lib.metu.edu.tr/upload/12605856/index.pdf

Also, by definition:

$$V_{max} = k_{cat}[E_T] \quad (2)$$

When the substrate concentration is much bigger than the Michaelis constant, [S] >> $K_M$ [7], which is the case, then:

$$V(t) = V_{max} \quad (3)$$

Considering the previous condition we equal the two previous equations in:

$$V(t) = k_{cat}[E_T] \quad (4)$$

*Heaviside function*

The total enzyme concentration that degrades the wall ($E_T$) is a function of the total Enzyme (E) which depends on the holes formed by the holin, using the critical concentration model approach, a modified Heaviside function ($H_{mod}$) was defined:

$$H_{mod}(E) = \begin{cases} 0 & if \quad E < H_c \\ 1 & if \quad E \geq H_c \end{cases} \quad (5)$$

Where E is the total amount of Endolysin/Holin added (molecules) and $H_c$ is the critical concentration of holin that causes holes in the cellular membrane (molecules).

This modified Heaviside function works as a unit step function that will allow discontinuity in the modeling, this discontinuity is used to model the holin behaviour and to associate both endolysin and holin in a single fused formula that predicts their activity.

The modified Heaviside function works as a switch, when the concentration of total molecules of Endolysin/Holin reaches the critical concentration of holin that a cell tolerates the function will start the enzymatic kinetics and catalytic approaches.

*Fick's First Law of Diffusion*

$$J = \frac{-D\ dC}{dx} \quad (6)$$

Where J is the flux of matter (a flow by unit of area), D is a diffusivity constant, dC is the difference of concentrations and dx is the length difference.

Fick's First Law of Diffusion is used to describe how matter reaches equilibrium between two zones with different concentrations of a specific molecule at steady state. Steady state means that both concentrations are considered to remain constant over the period of analysis. A better approximation

[7] Michaelis - Menten Kinetics. (n.d.). Retrieved from http://chemwiki.ucdavis.edu/Core/Biological_Chemistry/Catalysts/Enzymatic_Kinetics/Michaelis-Menten_Kinetics

for systems whose concentrations change is Fick's Second Law of Diffusion. For a first proposal the first law will be used since the analysis using the second law implies numerical methods[8].

Depending on the nature of the system the first law will take several forms. Holins form stable holes whose diameter and length are constant, therefore the area is constant. For systems with these characteristics the function takes this form after integration:

$$J = \frac{-D\,\Delta C}{\Delta x} \tag{7}$$

It is important to remember that a flux is a flow over unit of area. Then, a flow can be seen as a velocity and therefore as a first order derivative. This law is used to determine how fast the molecules of Endolysin/Holin will accumulate after the hole has been formed. The difference of concentration will become a driving force that will cause the Endolysin/Holin to reach the cell wall, its substrate. Also, since the most accepted notion is that holes do not expand, but several can be formed, then the number of holes will determine how much protein can reach the cell wall. With these considerations Fick's Law has been adapted to the project:

$$\frac{dE_T}{dt} = \frac{-D * \Delta C}{\Delta x} * A * n \tag{8}$$

where $dE_T/dt$ is the rate of change of the enzyme that has reached its substrate and is catalytically active, D is the diffusivity constant, A is the area of the hole, n is the number of holes, $\Delta x$ is the length of the hole, and $\Delta C$ is the difference of concentration of Endolysin/Holin.

This equation can be integrated to know how the amount of catalytically active enzyme changes over time. Also, $\Delta C$ is a number determined by the user because the initial concentration and amount added are known. Final concentration is 0 because there are no Endolysin/Holin between the cell membrane and the cell wall, while the initial concentration is E (the total enzyme previously mentioned), defined as the amount of Endolysin/Holin added to the system.

The function is integrated

$$\int dE_T = \int \frac{-D * \Delta C}{\Delta x} * A * n * dt$$

$$\int dE_T = \int \frac{-D * (E_f - E_i)}{\Delta x} * A * n * dt$$

$$\int dE_T = \int \frac{-D * (0 - E)}{\Delta x} * A * n * dt$$

$$\int dE_T = \int \frac{-D * (-E)}{x} * A * n * dt$$

and the following function is obtained since the initial conditions are $E_T(0) = 0$ and the integration constant C = 0

[8] Lecture 4: Fick's Second Law. (n.d.). Retrieved from http://www.eng.utah.edu/~lzang/images/lecture-4.pdf

$$E_T(t) = \frac{D * E * A * n}{x} * t \quad (9)$$

where D is the diffusivity constant, E is the total amount of Endolysin/Holin added to the system, A is the area of the hole, n is the number of holes, and x is the length of the hole.

*Fick's Second Law of Diffusion*

The second law is derived from the first law. Here, concentration changes can be studied in a more precise way because it is a function of time and position, therefore the changes of solute (enzyme in this case) can be predicted better.

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \quad (10)$$

This equation is very similar to the heat equation. It a partial differential equation which needs initial conditions and frontier conditions to obtain a satisfactory solution to a specific problem. The equation has infinite solutions, from which one is obtained using the described parameters.

In order to simplify the approach to the solution, the Thin Source solution[9] will be used. For this solution to work correctly it needs two considerations: the total amount of solute remains constant (the enzyme is not added several times) and the initial concentration of solute in the studied space is 0. For this case, the solution takes the following form

$$C(x,t) = \frac{E}{\sqrt{\pi D t}} e^{\frac{-x^2}{4Dt}} \quad (11)$$

where E is the initial concentration of solute (enzyme) and D is the diffusivity constant.

From this equation only one point is of interest: the end of the holin tunnel at a length *L*. Other points are not essential for the study because when the enzyme has finally passed through all the tunnel (it is at point *L*) it means that is has reached a region where substrate is available. Then

$$C(L,t) = \frac{E}{\sqrt{\pi D t}} e^{\frac{-L^2}{4Dt}} \quad (12)$$

The Thin Source solution takes into account that a small portion of material is going to be inserted between another material as shown in **Figure 3**, in the model we expect that the enzyme is placed just in one side of the bacterial wall, so modifications to equation (12) are needed in order to reflect this condition.

[9] Fick's Second Law. (2015). University of Cambridge. Retrieved from http://www.doitpoms.ac.uk/tlplib/diffusion/fick2.php

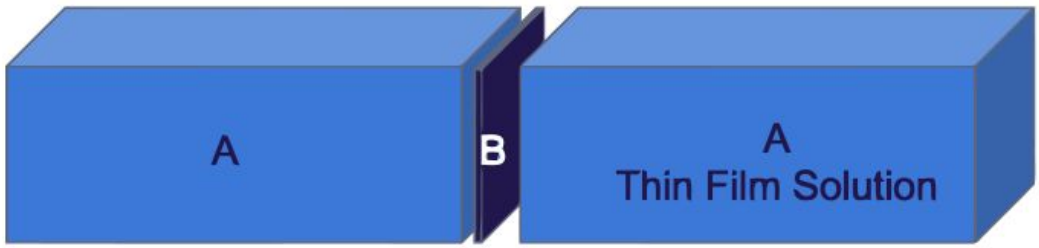


**Figure 3.** Thin film solution initial conditions.

The modification of the Thin film solution stands as the following equations.[10]

$$C\,(L,t) = \frac{E}{2\sqrt{\pi D t}} \sum_{i=1}^{n} \Delta\alpha_1 e^{\left[\frac{-(x-\alpha_i)^2}{4Dt}\right]}$$

Finally we solve the summation within an interval and obtain an error function, this error function is included within the modified thin film solution.

$$C(x,t) = \frac{E}{2}[1 + \mathrm{erf}\left(\frac{x}{2\sqrt{D\,t}}\right)] \tag{13}$$

*Model using Fick's First Law of Diffusion*

To form the first proposal model the previous three equations are taken into account: Michaelis - Menten in a condition where $[S] >> K_M$, a modified Heaviside function that will switch the function to the enzymatic mode once the critical $H_c$ concentration has been reached, and a function derived from Fick's First Law that will determine how fast the enzyme will reach its substrate.

$$V(t,E) = H_{mod} * \left(k_{cat}\left[\frac{D * E * A * n}{x}\right] t\right) \tag{14}$$

The model will determine the rate at which bonds are cleaved by the endolysin, which is a first order derivative. The parameter E is a constant defined by the user when the product is applied. This will allow a second integration to determine the number of bonds cleaved over time. The $H_{mod}$ function is omitted in the integration. The user should be careful in determining the amount E added so that it is greater or equal to $H_c$, in which the following integration will directly analyse the catalysis.

[10] Sanders, T. H. (s.f.). Lecture 67 - 4.14 Fick's Second Law - Modifications to the Thin Film Solution Retrieved from https://www.coursera.org/learn/material-behavior/lecture/nbxlY/4-14-ficks-second-law-modifications-to-the-thin-film-solution

$$\int V(t,E)\,dt = H_{mod} * \int \left(k_{cat}\left[\frac{D * E * A * n}{x}\right]t\right)dt$$

This integration will result in the M function that describes the amount of bonds of the cell wall cleaved over time by the endolysin. The time needed for complete lysis can be approximated with an estimate of cleaved bonds in the cell wall by other phages or lysozymes. For t=0 the number of bonds cleaved is 0, therefore C = 0 and

$$M(t,E) = H_{mod}(k_{cat}[\frac{D * E * A * n}{2x}]t^2 \tag{15}$$

*Model using Fick's Second Law of Diffusion (modified Thin - Film solution)*

The same considerations of the model with the first law can be applied. The enzyme concentration is given again by the diffusion law. The position *x* is substituted for *L* because that is the position of interest for the study. The catalytic velocity takes the following form:

$$V(t,E) = H_{mod} * k_{cat}\frac{E}{2}\left[1 + \text{erf}\left(\frac{L}{2\sqrt{D\,t}}\right)\right] \tag{16}$$

A second integration is necessary to obtain the M function previously defined. Again, the parameter E is considered constant and therefore the function can be integrated directly:

$$\int V(t,E)dt = H_{mod} * k_{cat}\frac{E}{2}\int \left[1 + \text{erf}\left(\frac{L}{2\sqrt{D\,t}}\right)\right]dt$$

After indefinite integration the equation M has the following form:

$$M(t,E) = H_{mod} * k_{cat}\frac{E}{2}\int \left[1 + \text{erf}\left(\frac{L}{2\sqrt{D\,t}}\right)\right]dt \tag{17}$$

$$M(t,E) = H_{mod} * \frac{kcat * E}{2} * [\left(\frac{L^2}{2D} + t\right)\text{erf}\left(\frac{L}{2\sqrt{D\,t}}\right) + \frac{L\,t\,e^{\frac{-L^2}{4\,D\,t}}}{\sqrt{\pi\,D\,t}} + t] \tag{18}$$

**Considerations**

The situation modeled by equations (15) and (17) take into account the moment when the enzyme has reached a cell. This means that the previous diffusion of the enzyme of the medium is not taken into account. The models assume that the medium has a homogeneous distribution of the enzyme. In the case where the enzyme needs to be analysed since its application its diffusion will be governed by Fick's Second Law, making the constant E a function with the characteristics of equation (14) for systems in one dimension (i.e. a tube) but not for other systems. This means that this function would need to be included in the integration processes.

## Conclusion

After all the enzymatic modeling it is reasonable to believe that a proper equation that involves both Holin and Endolysin behavior with the correct parameters was obtained. The use of Fick's second law provides more information on concentration based on position and time than the first law. Finally, a modified Thin layer solution has the advantage of easy to substitute equations, with the sole complication of the integration of the erf function.